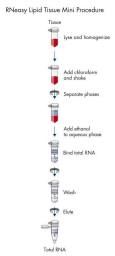
Dec 02, 2024

Bulk RNAseq extraction



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Protocol status: Working
We use this protocol and it's working

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Abstract

RNA extraction from frozen brain using Qiagen's RNeasy Lipid Tissue Mini kit.

Attachments

PDF

HB-0144-004_HB_RNY_L...

254KB

Materials

	Reagent	Manufacturer	Cat no.
	RNeasy Lipid Tissue Mini	Qiagen	74804
	Chloroform	Millipore Sigma	C2432
	Pure Ethanol	Millipore Sigma	E7023
	Qubit RNA Broad Range kit	Thermo Fisher Scientific	Q10210
	TissueRuptor II	Qiagen	9002755
	TissueRuptor II probes	Qiagen	990890
	RNase Zap	Thermo Fisher Scientific	AM9780
	RNase-Free DNase set	Qiagen	79254

Cutting Brain Tissue

1 Manually cut 40-50mg of frozen brain tissue on dry ice. Weigh and place back in -80 °C freezer until ready for extraction.

Note: do not let tissue thaw. Keep frozen at all times.

RNA extraction

2 Using the Qiagen RNeasy Lipid Tissue Mini kit, prepare reagents and materials for extraction in a chemical hood.

Note: use an RNase free environment and clean with RNase Zap.

3 Follow extraction steps outlined in Qiagen RNeasy Lipid Tissue Mini kit using the Tissue Ruptor II to homogenize frozen brain tissue.

3.1 Include on column DNase digestion step.

Note: keep extracted RNA on wet ice.

Quality Control

4 Using the Qubit RNA Broad Range assay kit, measure the concentration of extracted RNA using 2 µL of sample.

5 Aliquot desired amount of sample to continue with for sequencing (>500 ng) .

5.1 Optional: sequence with Illumina's TruSeq stranded riboreduced total RNA at the recommended 50M reads/sample.

Storing RNA

6 Snap freeze each RNA sample with liquid nitrogen and store in -80 °C freezer indefinitely.